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PC094599 Technical Abstract

Career goal: The goal of my career is to become an independent and productive scientist in the area of prostate cancer research. I am especially committed to improve the efficacy of current treatment modalities for advanced prostate cancer patients through translating my research into clinical applications.

Training program in Southern Illinois University School: Currently I am enrolled in Molecular Biology, Microbiology, and Biochemistry Graduate Program in Southern Illinois University. This is a multidisciplinary graduate program that enables students to be exposed to many aspects of modern biomedical research and to obtain broad knowledge base. The laboratory I have chosen to conduct my doctoral research is located in SimmonsCooper Cancer Institute (SCCI), a rapidly growing cancer center serving the rural areas of Illinois and neighboring states. Prostate cancer is one of two research focuses in SCCI. Basic scientists of prostate cancer training program include, but not limited to, Dr. Daotai Nie, Dr. Kounosuke Watabe, and Dr. Andy Wilber. Dr. Nie has close collaborations with clinician scientists in the program include Drs. Thomas Tartar (surgical oncologist), Brad Schwartz (surgical oncologist), Krishna Rao (medical oncologist) and Gregory Akers (pathologist). Through this training program, I am trained in basic, translational, and clinical aspects of prostate cancer research.

Background and significance: It is now increasingly accepted that cancer stem cells (CSCs, or tumor initiating cells) are responsible for tumor initiation. If cancer treatment kills most of cancer cells in the stage of transit amplifying and differentiation without killing the stem cells, the surviving cancer stem cells will eventually lead to recurrence of tumors. To eradicate cancer, we must learn more about the biology of cancer stem cells, their responses to treatments, and their role in tumor recurrence after treatment. In the preliminary studies, I found that Nanog, a transcription factor essential for self-renewal of embryonic stem cells, was expressed in prostate cancer cells, and further its expression was associated with tumor cells positive for stem/progenitor markers. Knockdown of Nanog reduced the ability of cancer cell to form tumors in an animal model. I further found that tumor cells with endogenous Nanog expression were particular resistant to chemotherapy. The data suggest that Nanog is associated with prostate cancer stem cells and Nanog may cause resistance toward chemotherapy.

Hypothesis: Nanog promotes resistance of prostate carcinoma cells toward chemotherapy and that Nanog, or its downstream effectors, should be targeted for eradication of tumorigenic prostate carcinoma cells.

Specific Aims: To test my hypothesis, the following specific aims are proposed: 1) To define the role of Nanog in resistance of prostate carcinoma cells toward chemotherapy. 2) To determine whether Nanog can be targeted to eliminate the chemoresistance of prostate cancer cells. 3) To elucidate the mechanism of Nanog-mediated chemoresistance.

Study Design: In aim 1, I will evaluate the responses of tumor cells enriched with endogenous Nanog expression or with Nanog overexpressed toward a range of chemotherapeutics. In Aim 2, I will knock down the expression of tumor Nanog and determine whether Nanog can be targeted to sensitize tumor cells toward chemotherapy. In aim 3, I will determine the expression and activities of ATP-binding cassette (ABC) transporters in cells enriched with Nanog expression, or with Nanog overexpressed, or with Nanog knocked down. The functional roles of ABC transporters identified in Nanog-mediated drug resistance will be determined using specific pharmacological inhibitors.

Impact: The proposed research will validate Nanog, or its downstream effectors, as a target of intervention to eliminate prostate cancer stem cells, advancing our goal to eradicate prostate cancer. Since a number of inhibitors of ABC transporters have been developed, the translation of the proposed studies into clinical applications will be greatly facilitated.

PC094599 Public Abstract

With determinations to do something about patients with advanced prostate cancer, I have been working on the potential role of cancer stem cells in resistance toward chemotherapy. The training environment in Southern Illinois University is excellent for my close interactions with members of prostate cancer research program as well as other talented researchers and clinicians. In the preliminary study, I found that Nanog, a transcription factor essential for self-renewal of embryonic stem cells, was expressed in prostate cancer cells, and further its expression was associated with tumor cells positive for stem/progenitor markers. Knockdown of Nanog reduced the ability of cancer cell to form tumors in an animal model. I further found that tumor cells with endogenous Nanog expression were particular resistant to chemotherapy. The data suggest that Nanog is associated with prostate cancer stem cells and Nanog may cause resistance toward chemotherapy. The objective of this proposal is to define the role of Nanog in resistance of prostate cancer cells toward chemotherapy, to determine whether Nanog can be targeted to sensitize tumor cells toward chemotherapy, and to identify the downstream effectors, especially a family of efflux transporters, called, ATP-binding cassette transporters, in Nanog-mediated chemoresistance. Various molecular, cellular, and pharmacological approaches will be employed to achieve the goals of the proposed studies. The proposed studies will validate whether we can target tumor Nanog to sensitize prostate cancer stem cells toward chemotherapy so that tumor recurrence or drug resistance can be eliminated. By performing and achieving the goals of the proposed research, the principal investigator will learn how to conduct cutting edge research that can be of high significance in the translational cancer research. The proposed research will boost my resolves to pursue a career in cancer research, with goal of becoming an independent cancer researcher in an academic setting.

PC094599 Impact Statement

Chemotherapy is the salvage treatment modality for patients with prostate cancer especially those at advanced stages. However, resistance to chemotherapy is a major barrier to eradicating prostate cancer cells. In the preliminary studies, I found that Nanog, a transcription factor essential for self-renewal of embryonic stem cells, is expressed in prostate cancer cells and its expression is associated with tumor cells with stem-like properties. Further, I found that prostate cancer cells with increased endogenous Nanog express presented as highly resistant to chemotherapeutics. It is hypothesized that as a determinant of stem cells, Nanog promotes resistance of prostate carcinoma cells toward chemotherapy and that Nanog, or its downstream effectors, should be targeted for eradication of tumorigenic prostate carcinoma cells. In this fellowship application, I propose to define the role of Nanog in resistance of prostate cancer cells toward chemotherapy, to determine whether Nanog can be targeted to sensitize tumor cells toward chemotherapy, and to identify the downstream effectors, especially a family of efflux transporters, called, ATP-binding cassette transporters, in Nanog-mediated chemoresistance. The proposed studies will validate whether we can target tumor Nanog to sensitize prostate cancer stem cells toward chemotherapy so that tumor recurrence or drug resistance can be eliminated. In addition, the proposed studies will identify and evaluate downstream effectors of Nanog, specifically ATP-binding cassette transporters, in chemoresistance. Since a number of inhibitors of ABC transporters have been developed, the translation of the proposed studies into clinical applications to eliminate drug resistant prostate cancer stem cells will be greatly facilitated.

PC094599 Focus Area Statement

The proposal is in the focus area of **Therapy**. We propose to identify new target, Nanog, or its downstream effectors, ATP-binding cassette transporters, in resistance to chemotherapy. The proposed studies will enable us to eliminate drug resistance prostate cancer stem cells so that prostate tumor recurrence or drug resistance can be eradicated.

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Introduction

It is now increasingly accepted that cancer stem cells (CSCs, or tumor initiating cells) are responsible for tumor initiation. If cancer treatment kills most of cancer cells in the stage of transit amplifying and differentiation without killing the stem cells, the surviving cancer stem cells will eventually lead to recurrence of tumors. To eradicate cancer, we must learn more about the biology of cancer stem cells, their responses to treatments, and their role in tumor recurrence after treatment. In the preliminary studies, I found that Nanog, a transcription factor essential for self-renewal of embryonic stem cells, was expressed in prostate cancer cells, and further its expression was associated with tumor cells positive for stem/progenitor markers. Knockdown of Nanog reduced the ability of cancer cell to form tumors in an animal model. I further found that tumor cells with endogenous Nanog expression were particular resistant to chemotherapy. The data suggest that Nanog is associated with prostate cancer stem cells and Nanog may cause resistance toward chemotherapy.

Based on the preliminary data, it was hypothesized that Nanog promotes resistance of prostate carcinoma cells toward chemotherapy and that Nanog, or its downstream effectors, should be targeted for eradication of tumorigenic prostate carcinoma cells. To test my hypothesis, the following specific aims are proposed:

- 1) To define the role of Nanog in resistance of prostate carcinoma cells toward chemotherapy.
- 2) To determine whether Nanog can be targeted to eliminate the chemoresistance of prostate cancer cells.
- 3) To elucidate the mechanism of Nanog-mediated chemoresistance.

BODY OF REPORT

Scientific portion:

Task 1. To define the role of Nanog in resistance of prostate carcinoma cells toward chemotherapy. (Months 1 – 12).

The studies in this task have been completed (please see the previous annual reports).

Task 2. To determine whether Nanog can be targeted to eliminate the chemoresistance of prostate cancer cells (Months 9 - 24).

The studies in this task have been completed (please see the previous annual reports).

Aim 3. To elucidate the mechanism of Nanog-mediated chemoresistance. (Months 18 - 36).

To determine the mechanisms involved in Nanog-mediated chemoresistance, we profiled genes involved in drug resistance, including drug metabolizing enzymes and transporters. As shown in **Figure 1**, MDR1 (ABCB1) and ABCG2 mRNA levels were significantly increased in DU145 cells enriched with Nanog1 promoter activities than in parental DU145 cells, while the expression of other ABC transporters were not significantly altered in DU145 cells enriched with Nanog expression.

Next we assessed the expression of MDR1 and ABCG2 at protein level. As shown in **Figure 2**, Western blot analysis revealed an increased level of p-glycoprotein (MDR1 or ABCB1) and ABCG2 in DU145 cells enriched with NANOG1 promoter activities, when compared to the parental control cells.

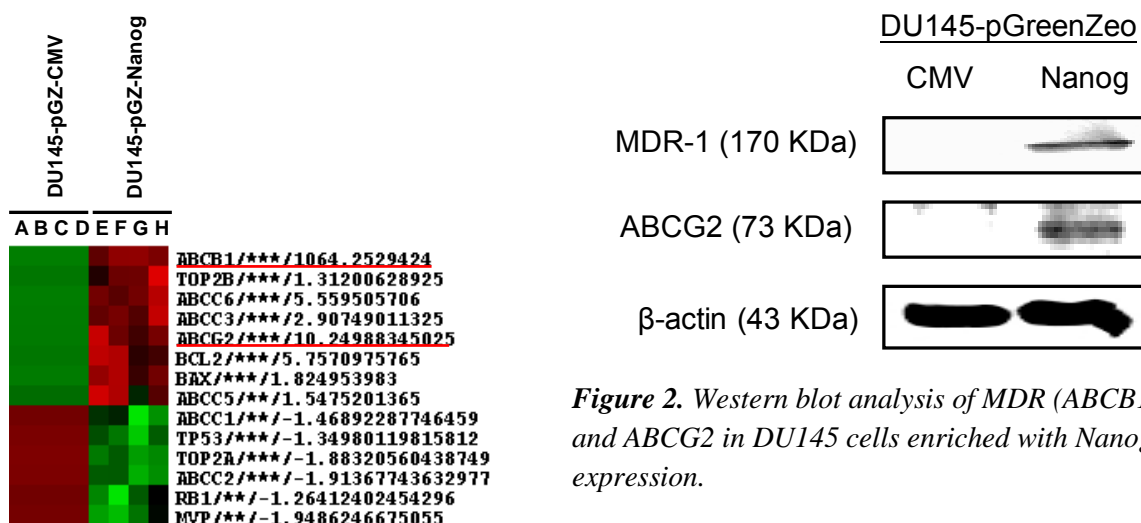


Figure 2. Western blot analysis of MDR (ABCB1) and ABCG2 in DU145 cells enriched with Nanog expression.

Figure 1. Gene expression patterns in DU145 cells with increased Nanog levels.

Immunocytochemical staining also revealed an increase in the surface expression of MDR1 as well as ABCG2 in DU145 cells enriched with NANOG1 promoter activities (**Figure 3**).

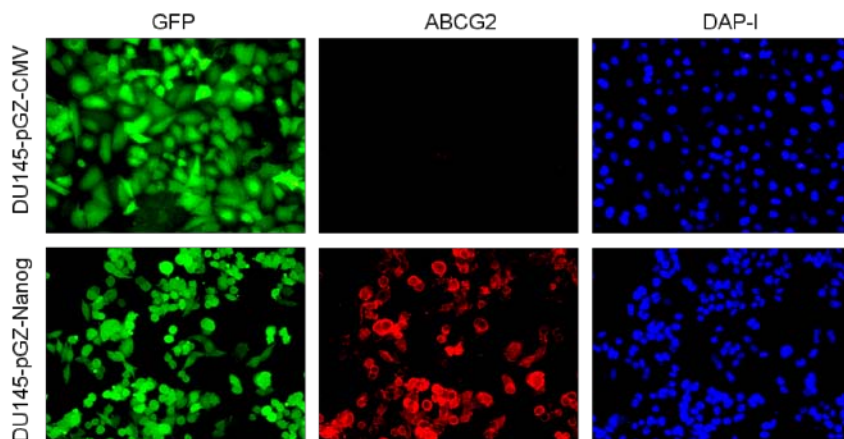


Figure3. Increased ABCG2 immunostaining in DU145 cells enriched with Nanog expression (DU146-pGZ-Nanog) when compared with control (DU145-pGZ-CMV).

To further determine the role of Nanog in the expression of MDR1 and ABCG2 in prostate cancer cells, we depleted Nanog via shRNA and then examined the subsequent changes in these two ABC transporters. Nanog depletion reduced the levels of MDR1 and ABCG2 expression in prostate cancer cells (Data not shown here_). The data further confirm the regulation of MDR1 and ABCG2 expression by Nanog.

Roles of MDR1 and ABCG2 in Nanog-mediated resistance

The increased expression of MDR1 and ABCG2 may confer the Nanog-expressing tumor cells with increased resistance toward chemotherapy. First we examined the effects of an inhibitor of ABCG2, FTC, on Nanog-mediated resistance. As shown in **Figure 4**, FTC treatment reduced, but not abolished, the resistance of Nanog-expressing cells toward vinblastine and doxorubicin, suggesting a partial contribution of ABCG2 in resistance of Nanog-expressing cells toward chemotherapeutics.

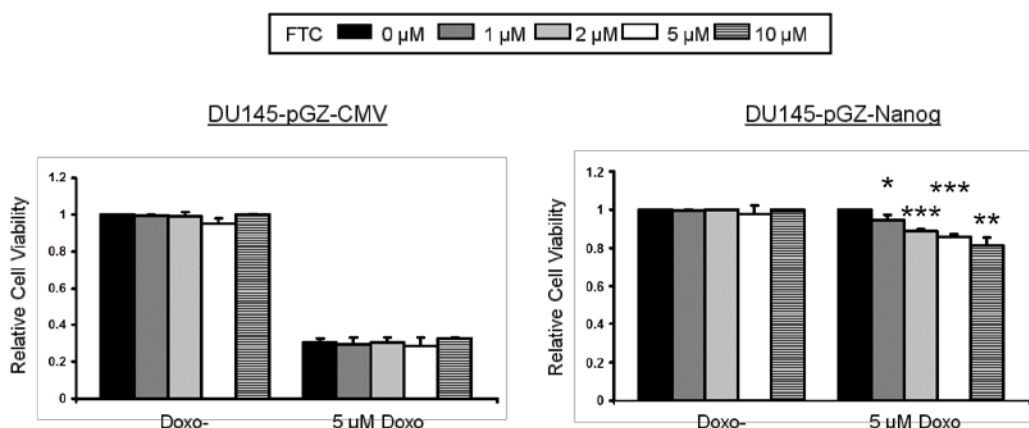


Figure 4. ABCG2 inhibitor FTC attenuated, but not abolished, the chemoresistance mediated by Nanog toward doxorubicin.

To determine whether MDR1 (ABCB1) also played a role in the Nanog-mediated resistance toward chemotherapy, we examined the effects of UIC2, a neutralizing antibody of MDR1, on Nanog-mediated resistance. As shown in **Figure 5**, UIC2 pretreatment reduced the increased resistance of DU145-Nanog cells toward Taxol. UIC2 pretreatment had minimal effects on the responses of DU145-CMV toward Taxol.

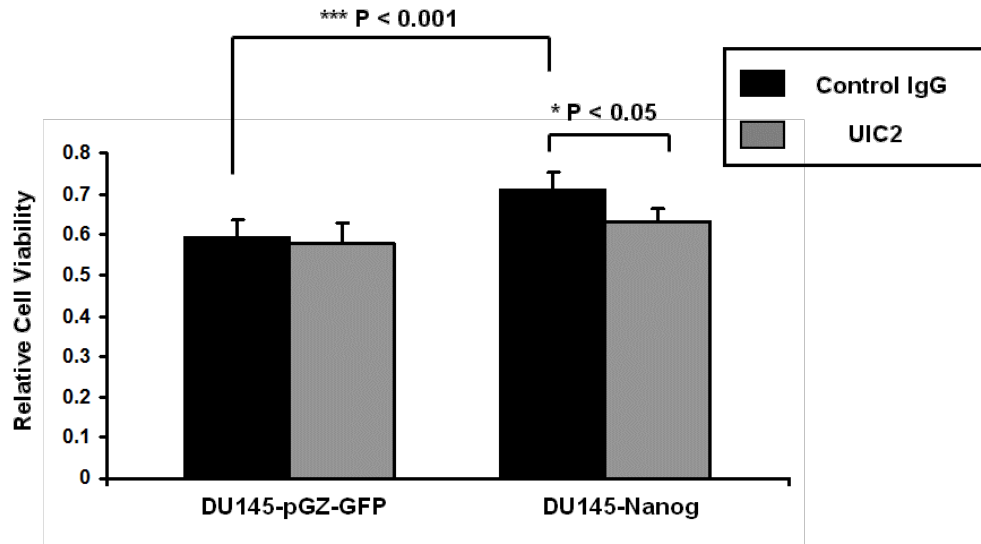
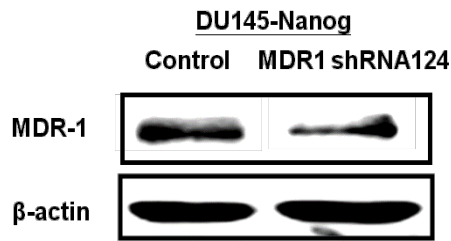


Figure 5. MDR1 neutralizing antibody UIC2 reduced the increased resistance of DU145-pGZ-Nanog cells toward Taxol.

To further test the role of MDR1 in Nanog-mediated resistance, we examined the effects of MDR1 depletion on Nanog-mediated resistance. As shown in **Figure 6**, depletion of MDR1 drastically reduced the resistance of Nanog-expressing cells toward Taxol and vinblastine, and to lesser extent doxorubicin, as evidenced by the reduced colony formation. The data suggest an important role of MDR1 in Nanog-mediated resistance.

A



B

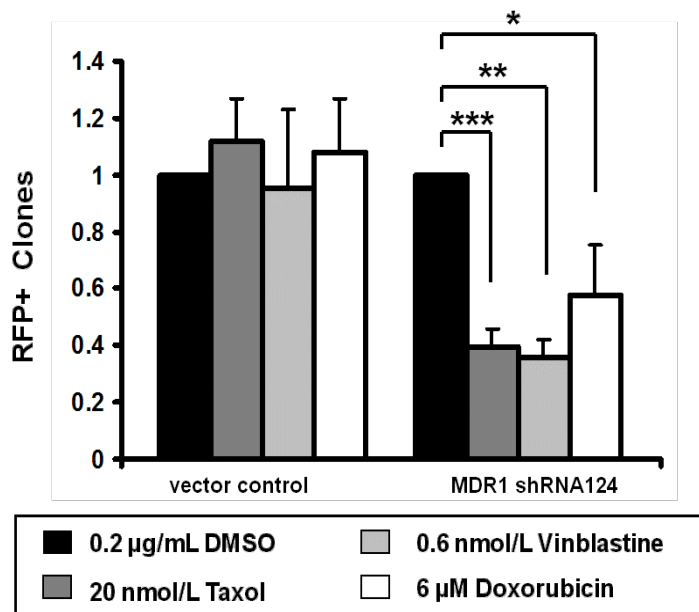


Figure 6. Depletion of MDR1 by shRNA sensitized DU145 cells enriched with Nanog toward chemotherapy. A, Western blot analysis of depletion of MDR1 by shRNA. B, Reduced colony formation of DU145-pGF-Nanog cells after depletion of MDR1 in responses to chemotherapy. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

The above data collectively suggest that the increased chemoresistance by DU145 cells enriched with Nanog activities is mediated by ABCG2 and MDR1.

Training portions

The PI, Ms. Hongmei Jiang, has had the following trainings:

A. Research-related training by learning all laboratory techniques required to complete the proposed studies, including, but not limited to: (Month 5 – 36).

Extraction of large plasmids more than 10 kb, cell culture, packaging of viral vectors, generation of stable cell lines with Nanog expressed or knocked down, FACS, evaluation of tumor cell responses to chemotherapy using MTS, trypan blue exclusion, and colony formation assays, Western blot, RNA isolation and cDNA synthesis, cloning, site-directed mutagenesis, and statistical analysis.

B. Non-research tasks important for PI's career development:

Ms. Jiang attended career development workshops sponsored by the 2013 AACR Annual Meeting held in Washington, DC in April, 2013.

B1. Oral presentations:

- 1) Presentations of research progresses in the lab meeting weekly. Ms Jiang has presented research findings in the lab meeting on weekly basis.
- 2) Presentations at student seminars. Ms. Jiang has given a seminar on her research findings in the spring. The audience is made up with students in the MBMB programs, faculty, and other interested researchers.
- 3) Presentation at scientific meetings. Ms Jiang presented the research findings in 2013 AACR Annual Meeting.

B2. Scientific writing skills:

- 1) Writing and submission of the annual progress report to DoD. (Every year)
- 2) Writing of research protocols or experimental approaches. (Every year)
- 3) Writing the first draft of manuscript to be submitted (2013- present)
- 4) Writing the first several chapters of dissertation (Dec. 2013 – present)

KEY RESEARCH ACCOMPLISHMENT and REPORTABLE OUTCOMES

Presentations:

Hongmei Jiang, Man-Tzu Wang and Daotai Nie. The Role of POU5F1B in Prostate Cancer. AACR 2013 Annual Meeting, Washington, DC, April 2013.

Hongmei Jiang, Man-Tzu Wang, and Daotai Nie. The Role of POU5F1B in Prostate Cancer. Simmons Cancer Institute 2013 Research Symposium, Springfield, IL, October 2013.

Abstracts published:

Hongmei Jiang, Man-Tzu Wang and Daotai Nie. The Role of POU5F1B in Prostate Cancer. Proc. Amer. Assoc. Cancer Res. 52 Late breaking: LB-281, 2013.

Articles published:

A manuscript is in preparation for publication.

Conclusions and significance (So what?):

Identification of key factors for tumor resistance to chemotherapy can lead to better strategy in cancer treatment. Our studies suggest that Nanog, a transcription factor essential for the self-renewal of embryonic stem cells, is expressed in tumorigenic cancer cells and further Nanog expression was enriched in the surviving fractions of tumor cells after chemotherapy. Knockdown of Nanog sensitized prostate cancer cells toward chemotherapy. Further we identified ABCG2 and MDR1 are key effectors for Nanog-mediated chemoresistance. Our studies suggest that ABCG2 and MDR1 can be targeted to improve the efficacy of chemotherapy of prostate cancer, especially those positive for Nanog.

APPENDICES

N/A

SUPPORTING DATA

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REFERENCES

N/A